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Cellular basis for electrocardiographic and arrhythmic manifestations of Andersen-Tawil syndrome (LQT7)

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Abstract

BACKGROUND Andersen-Tawil syndrome, a skeletal muscle syndrome associated with periodic paralysis and long QT intervals on the ECG, has been linked to defects in *KCNJ2*, the gene encoding for the inward rectifier potassium channel (I_{K1} .)

OBJECTIVES The purpose of this study was to examine the cellular mechanisms underlying the ECG and arrhythmic manifestations of Andersen-Tawil syndrome.

METHODS To investigate the effects of *KCNJ2* loss-of-function mutations responsible for Andersen-Tawil syndrome, we used barium chloride ($BaCl_2$) to inhibit I_{K1} in arterially perfused wedge preparation. Transmembrane action potentials (APs) were simultaneously recorded from endocardial, midmyocardial, and epicardial cells, together with a transmural ECG.

RESULTS $BaCl_2$ (1 to 30 μM) produced a concentration-dependent prolongation of the QT interval, secondary to a homogeneous prolongation of AP duration of the three cell types. QT interval was prolonged without an increase in transmural dispersion of repolarization (TDR). Low extracellular potassium (2.0 mM), isoproterenol (20 -50 nM), and an abrupt increase in temperature (36°C-39°C) in the presence of 10 μM $BaCl_2$ did not significantly increase TDR but increased ectopic extrasystolic activity. Early afterdepolarizations were not observed under any condition. Spontaneous torsades de pointes arrhythmias were never observed, nor could they be induced with programmed electrical stimulation under any of the conditions studied.

CONCLUSION Our results provide an understanding of why QT prolongation associated with Andersen-Tawil syndrome is relatively benign in the clinic and provide further support for the hypothesis that the increase in TDR, rather than QT interval, is responsible for development of torsades de pointes.

Keywords

Long QT syndrome; Sudden cardiac death; Arrhythmias; Ion channelopathy; Ventricle

Introduction

Studies have linked mutations in *KCNJ2*, which encodes the inward rectifier potassium channel I_{K1} , to Andersen-Tawil syndrome, also referred to as Andersen syndrome of the LQT7 form of the long QT syndrome.¹⁻⁶ This skeletal muscle syndrome is associated with periodic paralysis often linked to fluctuations in plasma potassium levels.^{2,7,8} Clinical studies indicate

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that Andersen-Tawil syndrome may be associated with arrhythmias, particularly when aggravated by other health problems such as infection.³

The cellular basis for the ECG and arrhythmogenic manifestations of Andersen-Tawil syndrome are not well defined. The present study was designed to develop and characterize an experimental model of Andersen-Tawil syndrome using canine left ventricular wedge preparations. Our protocols are designed to define the effects of reduced I_{K1} (using barium chloride [BaCl_2]) on transmural repolarization gradients, the morphology of the ECG T wave, the appearance of early afterdepolarization (EAD)-induced triggered activity, and the development of spontaneous and programmed electrical stimulation-induced torsades de pointes arrhythmias. We also assessed the modulatory influence of rate, temperature, adrenergic stimulation, and extracellular potassium levels.

Methods

Dogs weighing 20 to 30 kg were anticoagulated with heparin and anesthetized with pentobarbital 30 to 35 mg/kg IV. The chest was opened via left thoracotomy. The heart was excised, placed in cold (4°C - 10°C) Tyrode's solution, and transported to a dissection tray. Preparations with dimensions of $25 \times 10 \times 10$ mm to $32 \times 17 \times 15$ mm were dissected from the left ventricle. The tissue was cannulated via a diagonal branch of the left anterior descending coronary artery and perfused with cold Tyrode's solution. Unperfused tissue was removed carefully using a razor blade or fine dissecting scissors. The preparation was placed in a small tissue bath and perfused with Tyrode's solution of the following composition (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, NaHCO_3 20, CaCl_2 1.8, MgSO_4 1, and glucose 5.5, bubbled with 95% O_2 and 5% CO_2 ($37^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$). The perfusate was delivered to the artery by a roller pump (Cole Parmer Instrument Co., Niles, IL, USA). Perfusion pressure was monitored with a pressure transducer (World Precision Instruments, Sarasota, FL, USA) and maintained at 50 ± 5 mmHg by adjusting the perfusion flow rate. The preparations remained immersed in the perfusate, which was allowed to rise to a level at least 5 mm above the tissue surface to avoid a temperature gradient between the cut surface and epicardial and endocardial surfaces of the preparation. Ventricular wedge preparations were allowed to equilibrate in the tissue bath until they were electrically stable for 1 hour and stimulated with bipolar silver electrodes applied to the endocardial surface at a basic cycle length of 2,000 ms. A transmural ECG was recorded using silver electrodes placed in the Tyrode's solution bathing the preparation, 10 to 15 mm from the epicardial and endocardial surfaces, along the same axis as the transmembrane recordings. The QT interval was measured as the time between QRS onset and the point at which the line of maximal slope of the final segment of the T wave crossed the isoelectric line. Transmembrane APs were recorded simultaneously from the endocardial, midmyocardial, and epicardial sites using three separate floating microelectrodes (DC resistance 10-20 M Ω ; 3 mM KCl). Endocardial and epicardial APs were recorded from the endocardial and epicardial surfaces of the preparations at positions approximating the transmural axis of the ECG recording. Midmyocardial AP was recorded from the cut surface $20 \pm 5\%$ from the endocardium.⁹ Action potential duration (APD) was measured at 50% repolarization (APD₅₀) and 90% (APD₉₀) repolarization. Activation time was measured as the interval between the stimulus artifact and upstroke of AP. Transmural dispersion of repolarization (TDR) was defined as the difference between the longest and shortest repolarization times (activation time + APD₉₀) of transmembrane APs recorded across the wall.

Study protocols

We initially examined the concentration-dependent effects of BaCl_2 (1-30 μM), which led us to a BaCl_2 concentration of 10 μM for the Andersen-Tawil syndrome model. Baseline recordings were obtained after 1 hour of equilibration. AP and ECG characteristics were

evaluated at cycle lengths of 4,000, 2,000, 1,000, and 500 ms in the absence and presence of 10 μM BaCl_2 . We attempted to induce ventricular tachycardia (VT) with programmed electrical stimulation, up to two extrastimuli applied to the epicardial surface site of briefest refractoriness. In the case of single extrastimuli, S1-S2 was abbreviated under the refractory period was reached (roughly equivalent to APD_{90}). In the case of double extrastimuli, we used an S1-S2 of 180 or 200 ms and then abbreviated S2-S3 until the refractory period was reached.

To examine the influence of hyperthermia, we abruptly increased the temperature of the perfusate from 36°C to 39°C within 5 to 6 minutes in the presence of 10 μM BaCl_2 . We continuously recorded the ECG and APs of three cell types for at least 15 minutes at a basic cycle length of 2,000 ms.

To examine the influence of adrenergic modulation, we applied isoproterenol (20 -50 nM) for a 10-minute period to preparations pretreated with 10 μM BaCl_2 . In most preparations, transient increase in T-wave amplitude was observed 3 to 4 minutes after isoproterenol administration, just before the increase in frequency of ectopic premature beats or development of monomorphic tachycardia. We report data of the ECG and APDs of the tissue layers recorded 3 to 4 minutes after isoproterenol administration as the dynamic state and those recorded 8 to 10 minutes after isoproterenol administration as steady state.

To examine the influence of $[\text{K}^+]_o$, we altered the concentration of potassium from 2.0 mM (low) through 4.0 mM (normal) to 6.0 mM (high) in the presence of 10 μM BaCl_2 . Data were collected 8 to 10 minutes after introduction of low, normal, or high concentration of potassium at a basic cycle length of 2,000 ms.

Statistical analysis

Statistical analysis of the data was performed using the Student's t-test for paired data or one-way repeated analysis of variance (ANOVA) followed by Bonferroni test, as appropriate. Data are given as mean \pm SEM.

Results

Figure 1A shows the concentration-dependent effects of barium chloride. The QT interval and APD of three cell types display a similar concentration-dependent prolongation. $\text{I}_{\text{K}1}$ block caused very significant slowing of phase 3 of the AP, resulting in flattening and widening of the T wave. Under control conditions, epicardial repolarization was always coincident with the peak of the T wave, full repolarization of the M cell coincided with the end of the T wave, and Tpeak-Tend correlated with TDR. This relationship was disrupted following exposure to BaCl_2 . Although repolarization of the M cell coincided with full repolarization of the M cell, the peak of the T wave was no longer coincident with full repolarization of the epicardial AP. Thus, the Tpeak-Tend interval prolonged even though TDR remained largely unchanged or showed a tendency to decrease (Figures 1B and 1C). Consequently, the Tpeak-Tend interval no longer provided an accurate index of TDR.

Figure 2 shows typical changes observed in the configuration of the ECG and AP of the three cell types after exposure to 10 μM BaCl_2 , which we selected to characterize this model of Andersen-Tawil syndrome. BaCl_2 10 μM significantly prolonged the QT interval from 255 ± 8 ms to 312 ± 12 ms (22.5%) and increased Tpeak-Tend from 25 ± 4 ms to 40 ± 8 ms (55%). TDR remained unchanged (22 ± 3 ms vs 19 ± 3 ms), although Tpeak-Tend prolonged (Figure 1C). EADs were never observed in any cell types either in the absence or presence of BaCl_2 . Spontaneous polymorphic VT, such as torsades de pointes, was not observed either in the absence or presence 10 μM BaCl_2 and could not be induced using programmed electrical stimulation. Ectopic premature beats, arising from the deep subendocardium (endocardial or

Purkinje cells) were observed in 10 of 21 preparations after 10 μM BaCl_2 but were rarely observed under baseline conditions.

Figure 3 shows the rate-dependent effect of 10 μM BaCl_2 on the transmural ECG and AP characteristics of the three cell types. BaCl_2 10 μM caused a reverse rate-dependent prolongation of these repolarization parameters. QT interval increased from 226 ± 5 ms to 264 ± 8 ms (17%), from 249 ± 5 ms to 299 ± 8 ms (20%), from 259 ± 5 ms to 318 ± 8 ms (23%), and from 265 ± 5 ms to 332 ± 8 ms (25%) at cycle lengths of 500, 1,000, 2,000, and 4,000 ms, respectively. EADs were not observed in any cell type recorded even at a very long cycle length (4,000 ms). Extrasystoles, most abundant at a cycle length of 4,000 ms, diminished as pacing cycle length was abbreviated, completely disappearing at a cycle length of 500 ms.

Previous studies showed development of EAD activity and T-wave alternans following a sudden acceleration of rate under long QT conditions.^{10,11} This was not the case with QT prolongation secondary to I_{K1} inhibition with 10 μM BaCl_2 . Abrupt abbreviation of CL from 2,000 to 1,000 ms, from 2,000 to 500 ms, or from 1,000 to 500 ms failed to induce EADs or any form of repolarization alternans.

Previous studies also demonstrated the effect of hyperthermia in promoting the induction of EADs and producing a transient prolongation of APD under long QT conditions.¹² This was not the case with BaCl_2 -induced prolongation of the QT interval (Figure 4). In the presence of 10 μM BaCl_2 , an abrupt increase in perfusate temperature from 36°C to 39°C caused a sustained abbreviation of the QT interval and APD of all three cell types. Table 1 lists average data of ECG and AP parameters. Of note, TDR decreased significantly at the higher temperature. The abrupt increase in perfusate temperature caused a similar abbreviation of APD in the absence and presence of 10 μM BaCl_2 . Torsades de pointes was not observed under these conditions, nor could it be electrically induced.

In the LQT1 and LQT2 variants of the long QT syndrome, β -adrenergic stimulation produces a biphasic effect on APD, a sharp increase in TDR, and, in some cases, induction of EADs.^{13,14} In this Andersen-Tawil syndrome model of long QT, isoproterenol (20 -50 nM) produced sustained abbreviation of the QT interval and APD of three cell types, no change in TDR, and no induction of EADs (Figure 5). On the other hand, extra-systolic activity was greatly enhanced by isoproterenol (20 -50 nM), in some cases leading to the development of a slow monomorphic tachyarrhythmia with cycle length of 470 ± 20 ms. VT was observed within 4 to 6 minutes after isoproterenol administration in eight of 15 preparations tested. Preparations in which this slow idioventricular rhythm developed in response to isoproterenol were excluded from further analysis. The QTpeak interval abbreviated more than QTend, resulting in prolongation of Tpeak-Tend, despite the fact that TDR remained unchanged. Neither EADs nor polymorphic tachyarrhythmia developed spontaneously, nor could torsades de pointes be induced with programmed electrical stimulation. These effects of isoproterenol were completely suppressed in four preparations pretreated with the beta-blocker propranolol (1 μM).

Given that plasma potassium levels are known to affect the course of the disease, we examined the effect of lowering and increasing $[\text{K}^+]_o$ in another experimental series. Figure 6 illustrates the effect of extracellular potassium on AP and ECG parameters in a canine left ventricular wedge preparation pretreated with 10 μM BaCl_2 . The QT interval abbreviated progressively as potassium concentration was increased from 2 to 6 mM. The slope of terminal portion of phase 3 of the AP steepened in all cases, leading to a progressive increase in the amplitude of the T wave. Increasing extracellular potassium abbreviated the APD of the epicardial cell more than that of the M and endocardial cells, leading to amplification of TDR. A shift from normal

(4 mM) to low (2 mM) $[K^+]_o$ resulted in a dramatic increase in extra-systolic activity, whereas the increase to 6 mM $[K^+]_o$ totally suppressed ectopic activity.

Discussion

Cellular and ionic basis for Andersen-Tawil syndrome

Andersen-Tawil syndrome is a rare skeletal muscle disorder often associated with prolongation of the QT interval on the ECG.^{2,3,7,8,15} The syndrome also has been referred to as Andersen syndrome and the LQT7 form of the long QT syndrome.² Patients with Andersen-Tawil syndrome typically present with the triad of periodic paralysis, cardiac arrhythmias, and developmental dysmorphisms. Studies have linked Andersen-Tawil syndrome to mutations in the potassium channel gene *KCNJ2*, which encodes the inward rectifier potassium channel Kir2.1 or I_{K1} . Andersen-Tawil syndrome-associated mutations in *KCNJ2* caused dominant-negative suppression of Kir2.1 channel function.^{1-6,16} Pharmacologically, barium is considered the most selective blocker of inward rectifier potassium current I_{K1} .¹⁷⁻¹⁹ In the present study, we used $BaCl_2$ to block I_{K1} and thus create an *in vitro* cardiac model of Andersen-Tawil syndrome.

$BaCl_2$ at concentrations from 1 to 30 μM induced a 3.8% to 40.0% prolongation of the QT interval, covering the full range of QT prolongation observed in patients with Andersen-Tawil syndrome. The median prolongation of QT interval reported in a large cohort of patients with Andersen-Tawil syndrome is 4.8% (440 [28] in Andersen-Tawil syndrome vs 420 [20] in controls; median [interquartile range]).²⁰

$BaCl_2$ 10 μM prolonged the QT interval by $22\% \pm 3\%$, compatible with other experimental models of potassium channel mutations (LQT1 and LQT2).^{13,21} I_{K1} is present in all ventricular myocytes and shows strong inward rectification; essentially no current flows through these channels at potentials positive to -40 mV.^{18,22} I_{K1} is essential for the maintenance of a stable resting potential and contributes importantly to final repolarization of the AP. The repolarization process is determined by a balance between inward and outward currents, and any increase in inward current or decrease in outward current results in prolongation of APD. Computer simulation and viral gene transfer studies have demonstrated a prolongation of the APD and a depolarizing shift of the resting membrane potential as a result of I_{K1} suppression.^{23,24} To our knowledge, ours is the first study to assess the differential effects of I_{K1} block on the AP of the three predominant cell types composing the ventricular myocardium.

Inheritance of Andersen-Tawil syndrome is autosomal dominant, although penetrance of the disease is highly variable, as is disease expression and severity. Patients with Andersen-Tawil syndrome having the heterozygous mis-sense mutation R67W in *KCNJ2* have been found to display nonspecific ECG abnormalities but no QT prolongation, despite a history of syncope and frequent ventricular premature beats.⁶ Biophysical characterization of R67W demonstrated loss of function and a dominant-negative effect on Kir2.1 current. In contrast to the clinical experience, our results demonstrate that I_{K1} block consistently prolongs APD and QT interval. These observations point to an important role of modifier genes in the ECG, arrhythmic, physical, and skeletal muscle manifestations of the syndrome.

In contrast to other long QT syndromes, sudden death occurs infrequently in patients with Andersen-Tawil syndrome.^{2,5} The relatively benign course of the disease is consistent with our inability induce torsades de pointes in the present model. This is in contrast to LQT1 (I_{Ks} block), LQT2 (I_{Kr} block) and LQT3 (augmented late I_{Na}) models of long QT developed using the wedge preparation, in which a large increase in TDR permits induction of torsades de pointes.²⁵ The development of frequent extrasystoles in the wedge model of Andersen-

Tawil syndrome is concordant with the high incidence of ectopic activity observed in the clinic, most likely as a result of enhanced automatic pacemaker activity in the Purkinje system. This manifestation is exaggerated in the presence of hypokalemia, in the experimental model, as it is in patients with the syndrome. Elevation of $[K^+]_o$ to 6 mM completely suppressed ectopic activity in our wedge preparation, likely via its actions in augmenting I_{K1} . Arrhythmic expression of Andersen-Tawil syndrome in the clinic includes bidirectional VT and slow polymorphic VT,^{2,7,8} likely due to automaticity arising at two or more sites simultaneously. We did not observe this in our model, possibly because of the limited size of the wedge preparation.

Isoproterenol increased ectopic activity as well. Unlike experimental models of LQT1 and LQT2,¹³ β -adrenergic influence did not amplify TDR or induce EADs in our experimental model of Andersen-Tawil syndrome.

Our wedge model of Andersen-Tawil syndrome displays a prolongation of the downslope of the T wave and a slurring of its terminal portion, thus closely mimicking the ECG manifestation of Andersen-Tawil syndrome in the clinic. Slurring of the terminal portion of the T wave is the result of slurring of the terminal portion of phase 3 of the AP. Whereas in LQT1-3 a prolongation of the interval between Tpeak and Tend is in proportion to the increase in TDR, this is not the case in the Andersen-Tawil syndrome model. This model clearly demonstrates that when an increase in Tpeak-Tend is caused by a reduction in the slope of phase 3, as with I_{K1} inhibition, it is not associated with an increase in spatial dispersion of repolarization.

Study limitations

As with any experimental study, extrapolation of our results to the understanding of the clinical syndrome must be approached with some caution. $BaCl_2$ is the agent most frequently used to selectively block I_{K1} ²⁶, but it is unlikely to mimic precisely the effects of KCNJ2 mutations. Barium is known to interfere with calcium-mediated inactivation of L-type calcium channels and thus to prolong the open time of the channel, which could contribute to APD and QT interval prolongation.²⁷ Because APD prolongation secondary to I_{Ca} augmentation is accompanied by a prominent augmentation of TDR (unpublished observation), the lack of TDR amplification with $BaCl_2$ suggests that QT prolongations is largely due to I_{K1} inhibition rather than I_{Ca} augmentation.

Barium-induced block of I_{K1} has been shown to exhibit time-dependent and voltage-dependent block and unblock capable of inducing slow diastolic depolarization.²⁸ This characteristic of drug binding may contribute to increased automaticity via a mechanism other than pure inhibition of I_{K1} .

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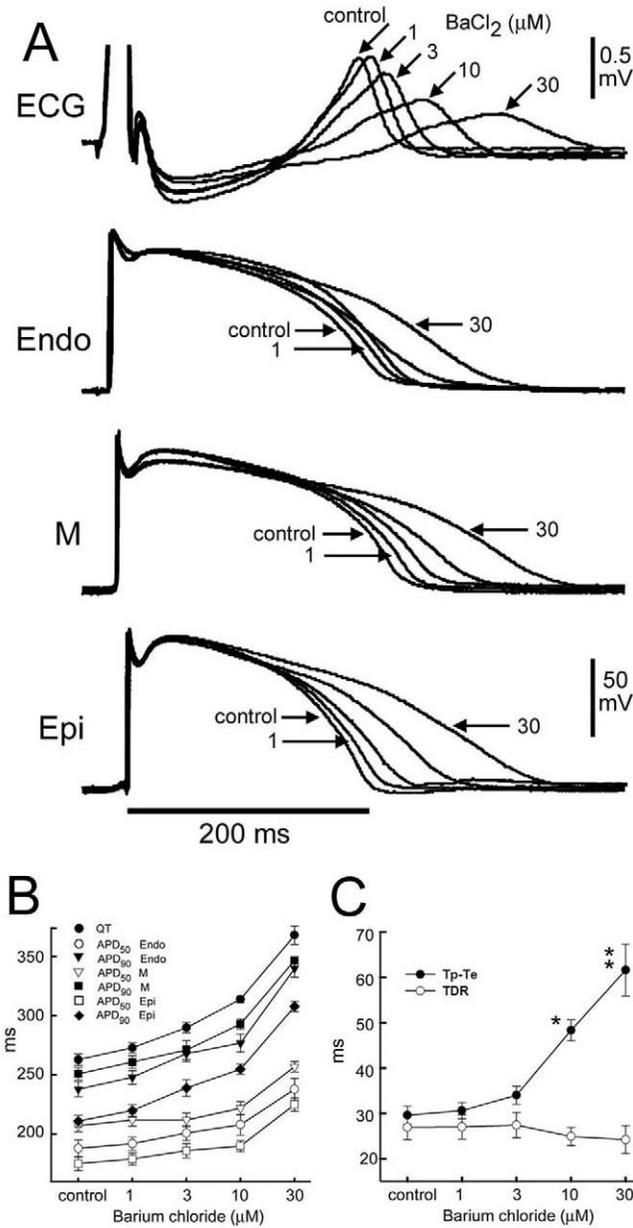


Figure 1. Dose-dependent effect of barium chloride on trans-membrane and ECG activity in canine left ventricular wedge preparation. Superimposed action potentials (APs) recorded simultaneously from endocardial (Endo), midmyocardial (M), and epicardial (Epi) cells, together with transmural ECG (A). Composite data of dose-dependent effects of barium chloride 1 to 30 μM on QT interval, APD₅₀, and APD₉₀ in endocardial (Endo₅₀, Endo₉₀), midmyocardial (M₅₀, M₉₀), and epicardial (Epi₅₀, Epi₉₀) (B), on Tpeak-Tend, and on transmural dispersion of repolarization (TDR) (C). Basic cycle length = 2,000 ms. n = 7. **P* < .05; ***P* < .01 vs control. BCL = basic cycle length.

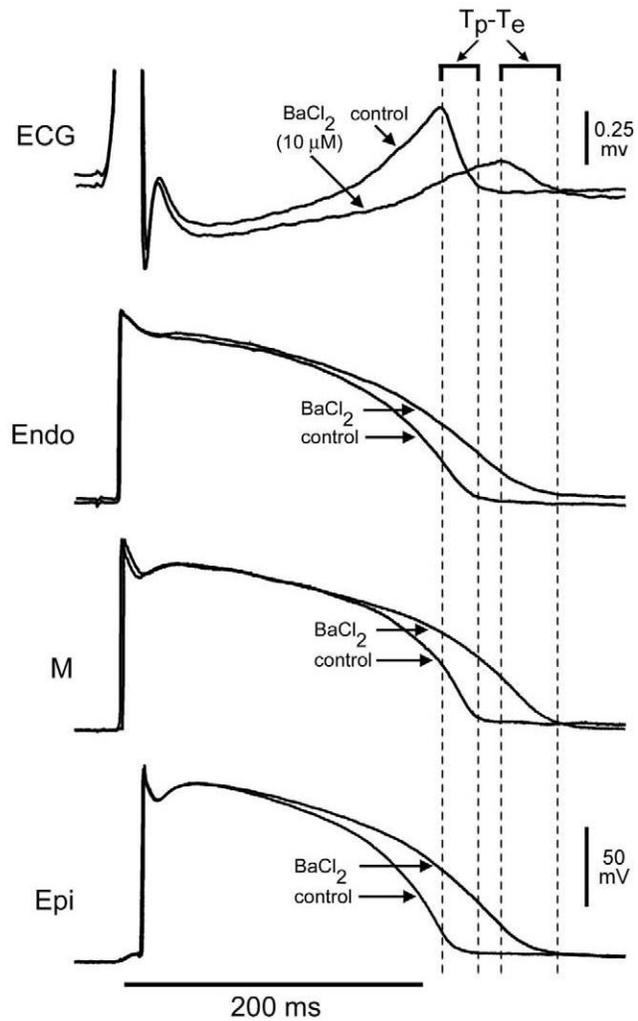


Figure 2. Representative configuration of action potentials of three cell types and ECG characteristics. Endocardial (Endo), midmyocardial (M), and epicardial (Epi) APs were simultaneously recorded with transmural ECG under control conditions and in the presence of 10 μM BaCl_2 .

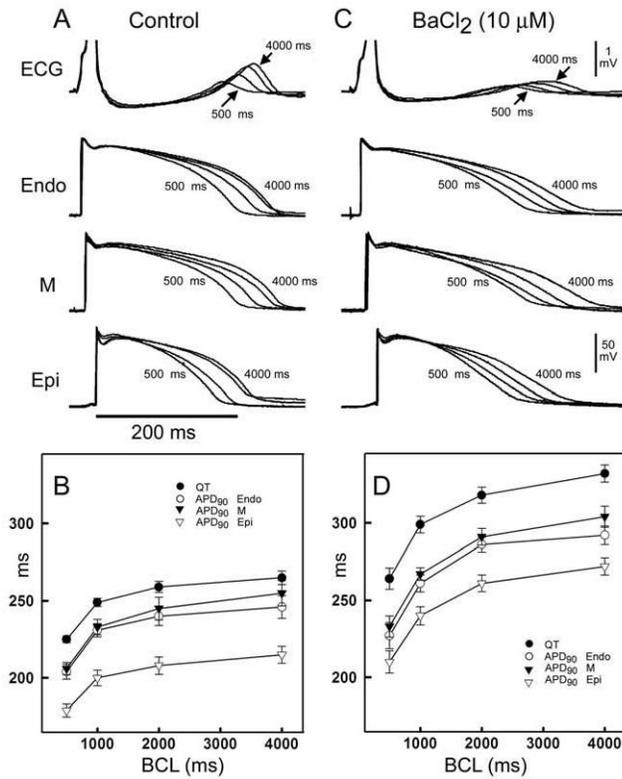


Figure 3.

Rate-dependent changes of action potential (AP) characteristics and QT interval under control condition and in the presence of 10 μM BaCl₂. Each trace shows superimposed APs recorded simultaneously from endocardial (Endo), midmyocardial (M), and epicardial (Epi) cells together with a transmural ECG at cycle lengths ranging from 500 to 4,000 ms. **A:** Control. **B:** Composite data of rate-dependent changes under control conditions. **C, D:** Recorded in the presence of 10 μM BaCl₂. n = 8. Endo = endocardial APD₉₀; Epi = epicardial APD₉₀; M = midmyocardial APD₉₀; QT = QT interval.

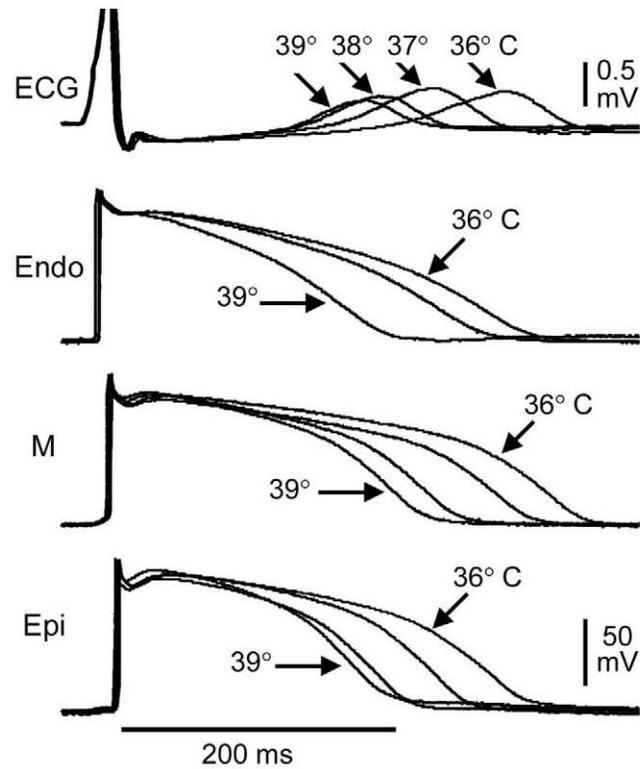
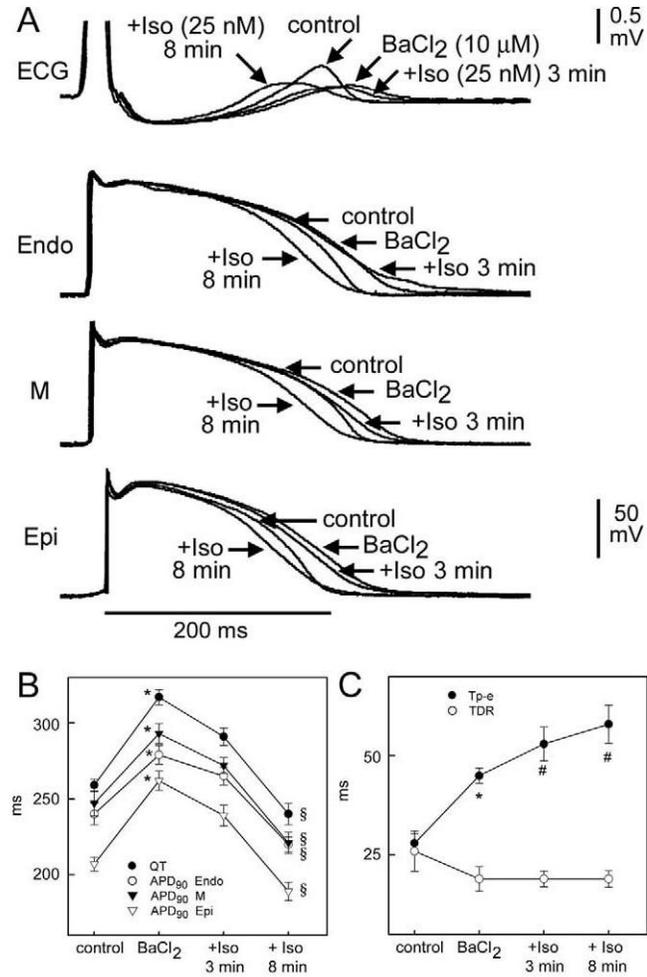


Figure 4.

Warming-induced QT and action potential (AP) abbreviation in arterially perfused left ventricular wedge preparation pretreated with 10 μ M BaCl₂. APs were recorded simultaneously from endocardial (Endo), midmyocardial (M), and epicardial (Epi) cells together with transmural ECG during a rise in perfusate temperature from 36°C to 39°C within 4 minutes (basic cycle length = 2,000 ms). Warming of the perfusate gradually and similarly abbreviates the APD of three cell types and the QT interval, resulting in reduction of Tpeak-Tend and transmural dispersion of repolarization (TDR). The tracings represent recordings of the response at 36°C, ~37°C and 38°C, and 39°C. Note that endocardial AP failed to record at ~38°C, so that the tracing is not shown.

**Figure 5.**

A: Effect of isoproterenol. Endo = endocardial cell; Epi = epicardial cell; M = midmyocardial cell. **B, C:** Composite data of effect of isoproterenol (20 or 25 nM) in the LQT7 model. BaCl₂ 10 μM produced a similar prolongation of action potential duration (APD) of endocardial (Endo), midmyocardial (M), and epicardial (Epi) cells. Isoproterenol in the continuous presence of 10 μM BaCl₂ caused a sustained abbreviation of APD of three cell types without significant change in transmural dispersion. Basic cycle length = 2,000 ms. n = 8. Endo = endocardial APD₉₀; Epi = epicardial APD₉₀; M = midmyocardial APD₉₀; QT = QT interval. **P* < .05 vs control; †*P* < .01 vs barium 10 μM; #No significant difference vs 10 μM BaCl₂.

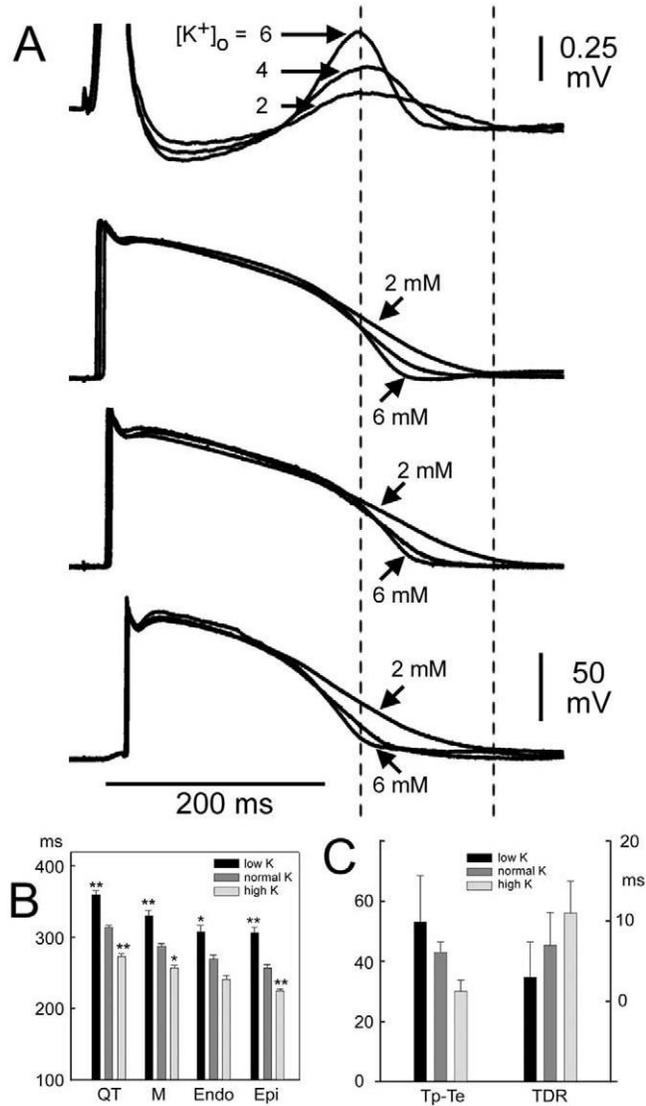


Figure 6. Effect of extracellular potassium level in the presence of $10 \mu\text{M BaCl}_2$. **A:** Superimposed action potentials simultaneously recorded from endocardial (Endo), midmyocardial (M), and epicardial (Epi) cells together with a transmural ECG at potassium concentrations of 2.0, 4.0, and 6.0 mM. **B, C:** Composite data of change in potassium level to low K (2.0 mM), normal K (4.0 mM), and high K (6.0 mM). Basic cycle length = 2,000 ms. $n = 7$. * $P < .05$ vs normal K; ** $P < .01$ vs normal K.

Table 1

Effect of temperature on action potential and ECG parameters in the presence of barium 10 μ M. Data were collected at 36°C and 5-6 min after an abrupt increase in perfusate temperature to 39°C (n = 8)

	QT	Tp-Te	TDR	APD ₅₀ Endo	APD ₉₀ Endo	APD ₅₀ M	APD ₉₀ M	APD ₅₀ Epi	APD ₉₀ Epi
36°C	337± 3.8	51± 3.6	27± 4.6	223± 4.9	302± 7.1	239± 5.8	310 ± 4.8	204± 8.5	268 ± 5.9
39°C	252 ± 4.7	44 ± 3.3	17± 3.7	150± 7.9	214± 8.2	174 ± 9.9	223± 4.2	142 ± 2.7	195± 2.8
% Δ	-25.0	-14.9	-36.7	-32.6	-29.2	-27.1	-27.9	-30.1	-27.4